

Supplementary Information

Characterization of Structural and Functional Domains of the Anillin-related Protein Mid1p that Contribute to Cytokinesis in Fission Yeast

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Running Head: Mid1p domains in *S. pombe* cytokinesis

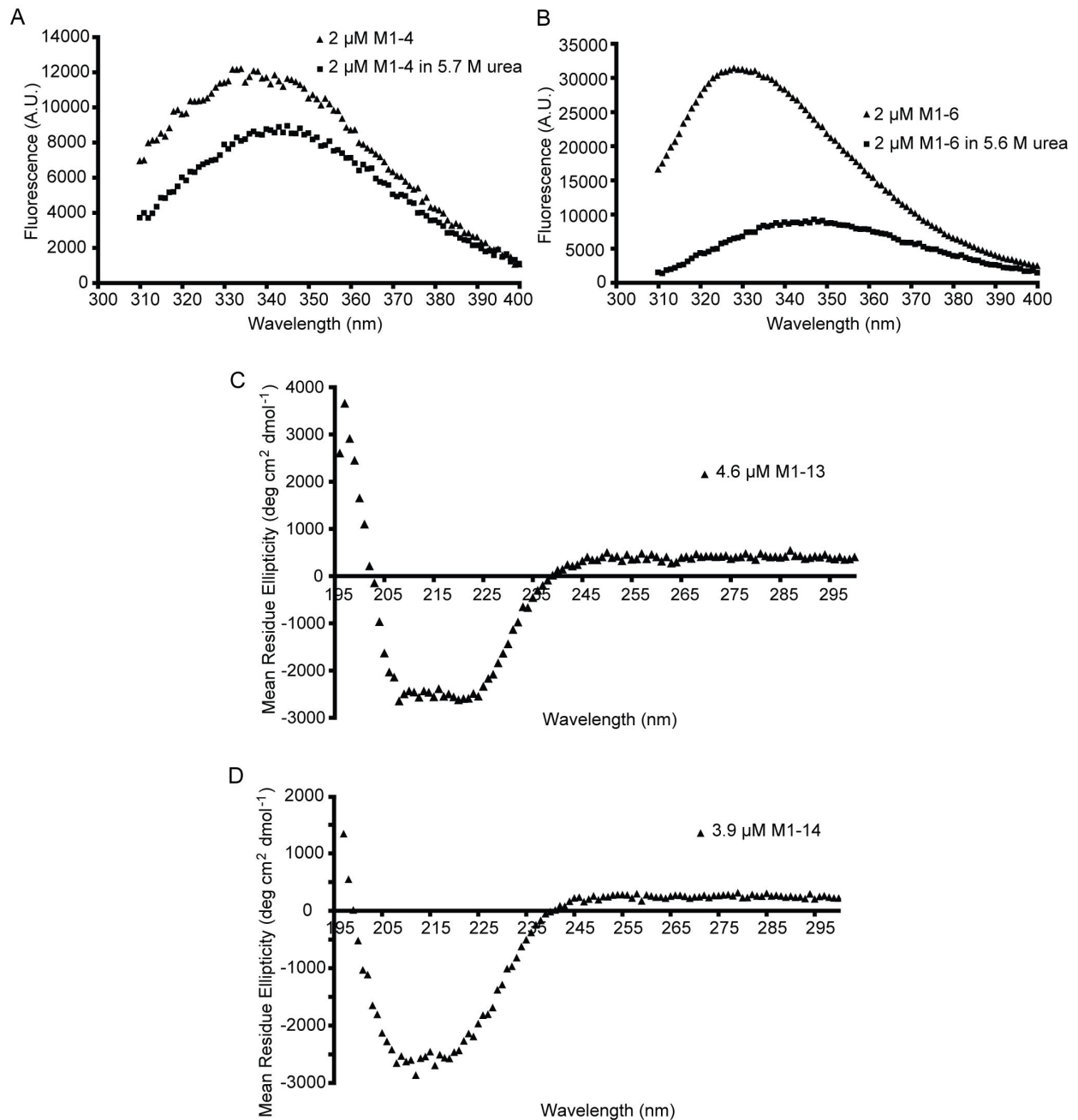


Figure S1 (related to Figure 1). Secondary and tertiary structure of soluble Mid1p domains. (A-B) Fluorescence emission spectra of purified, recombinant domains of Mid1p with excitation at 280 nm. (A) 2 μ M M1-4 in 50 mM sodium phosphate, 300 mM NaCl pH 8.0 (\blacktriangle) alone or (\blacksquare) with 1 mM DTT, 0.5 mM EDTA, 5.7 M urea. (B) 2 μ M M1-6 in 50 mM sodium phosphate, 300 mM NaCl pH 8.0 (\blacktriangle) alone or (\blacksquare) with 1 mM DTT, 0.5 mM EDTA, 5.6 M urea. (C-D)

Circular dichroism spectrum of recombinantly purified (C) 4.6 μ M M1-13 at 25 °C in 10 mM Tris, 150 mM NaCl, 1 mM TCEP pH 7.3. (D) 3.9 μ M M1-14 at 25 °C in 10 mM Tris, 50 mM NaCl, 1 mM TCEP pH 8.0.

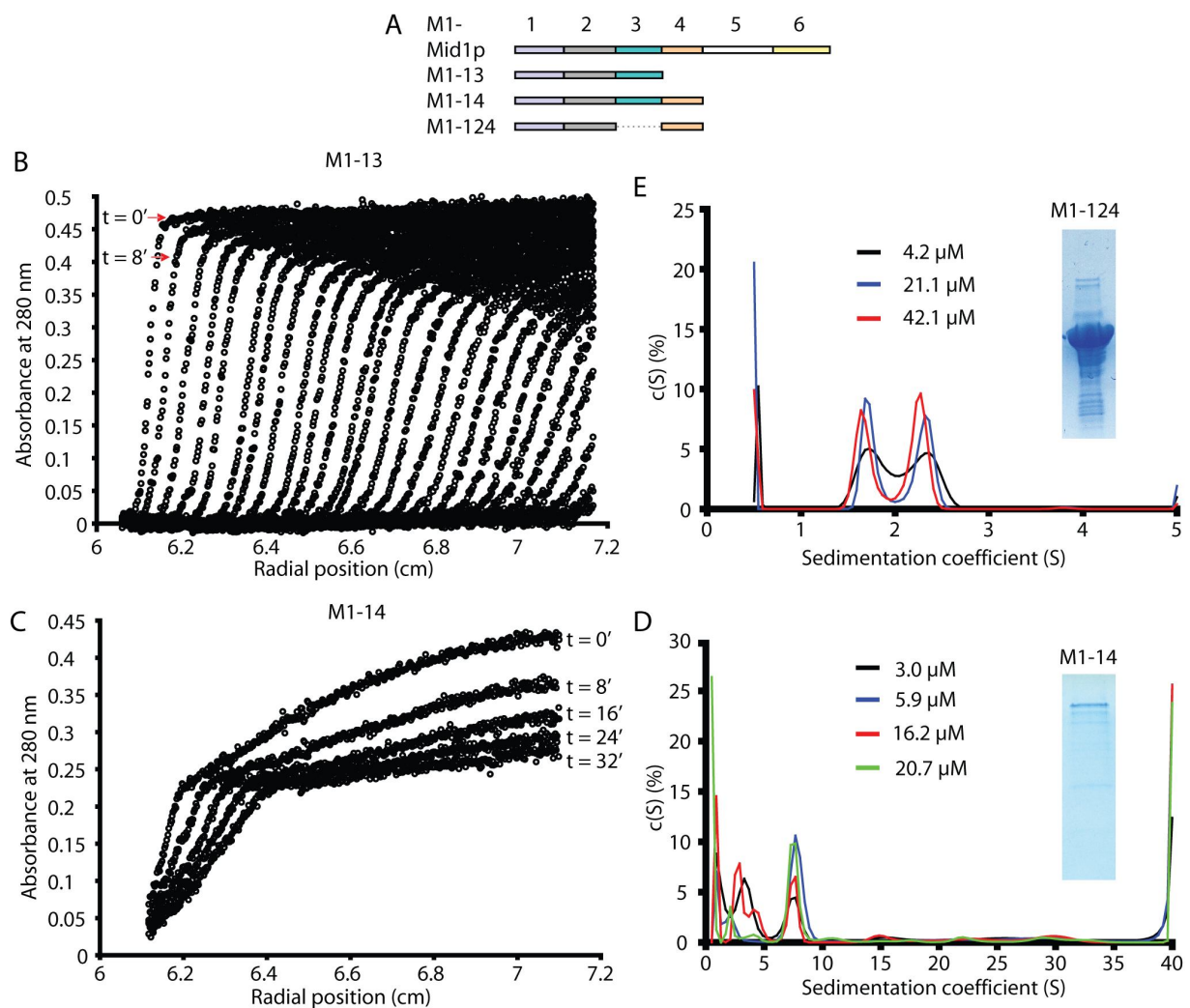


Figure S2 (related to Figure 2). Analysis of Mid1p constructs by sedimentation velocity analytical ultracentrifugation. (A) Maps of Mid1p constructs. Broken lines represent domain deletions. (B-C) Time courses of sedimentation at 20 °C at 42,000 rpm in 20 mM Tris, 150 mM NaCl, 1 mM TCEP pH 8.0. The plots show absorbance at 280 nm as a function of radial position from the axis of rotation in the ultracentrifuge cell at ~8 min intervals. (B) 23 μM M1-13 (residues 1-452). (C) 16.2 μM M1-14 (residues 1-578). (D-E) Distributions of sedimentation coefficients assuming a continuous $c(S)$ distribution. Bin width: one-hundredth of the range of sedimentation coefficients plotted. Conditions: 42,000 rpm in 20 mM Tris, 150 mM NaCl, 1 mM TCEP pH 8.0 at 20 °C. Insets show SDS-PAGE of M1-14 and M1-124. (D) M1-14 at concentrations of (black) 3.0 μM , (blue) 5.9 μM , (red) 16.2 μM and (green) 20.7 μM . (E) M1-124 at concentrations of (black) 4.2 μM , (blue) 21.1 μM , and (red) 42.1 μM .

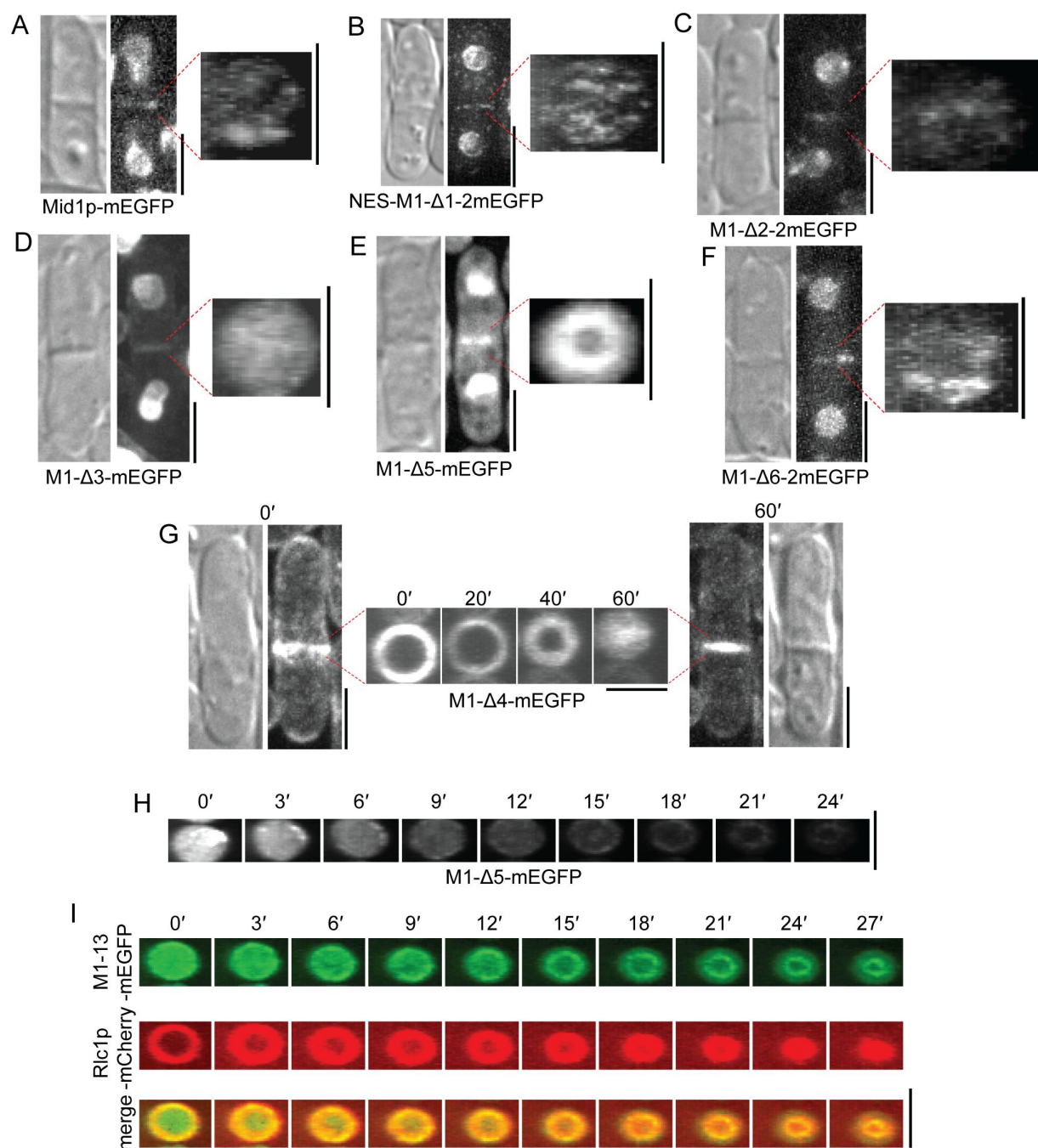


Figure S3 (related to Figure 4). Contributions of Mid1p domains to localization of Mid1p near septa. (A-F) Cells with a septum illustrated by DIC images, maximum intensity projections of stacks of fluorescence confocal sections and (to the right) projections in the YZ-plane of the equatorial areas indicated by the broken red lines. (A) A wild type cell expressing Mid1p-mEGFP from the *mid1* locus. (B-F) $\Delta mid1$ cells expressing Mid1p constructs: (B) NES-M1- $\Delta 1$ -

2mEGFP from the *mid1* locus, (C) M1-Δ2-2mEGFP from the *mid1* locus, (D) M1-Δ3-mEGFP from a cloned *mid1* promoter in the *leu1* locus, (E) M1-Δ5-mEGFP from a cloned *mid1* promoter in the *leu1* locus, (F) M1-Δ6-2mEGFP from the *mid1* locus. (G) Localization of M1-Δ4-mEGFP to the contractile ring and near septum when expressed from a cloned *mid1* promoter in the *leu1* locus in a *Δmid1* cell. The central panel is a time series of YZ-plane image projections of the equatorial area of the cell indicated by the broken red lines. (H-I) Time series of YZ-plane image projections of the equatorial area of individual *Δmid1* cells expressing Mid1p constructs: (H) M1-Δ5-mEGFP from a cloned *mid1* promoter in the *leu1* locus, (I) M1-13-mEGFP from a cloned *mid1* promoter in the *leu1* locus and Rlc1p-mCherry from the *rlc1* locus. Scale bars, 5 μm.

Table S1. Bacterial expression screen statistics.

Screen #	Template for construction of cDNA fragment library	Total number of colonies screened (approximate)	Total number of distinct Mid1p fragments identified	List of Mid1p fragments identified (amino acid residues)
1	Mid1 (full length)	14500	6	6-149 6-251 6-209 266-324 489-578 850-915
2	Mid1 (full length)	7750	0	-
3	Mid1 (amino acids 309-920)	3655	2	314-452 798-915
4	Mid1 (amino acids 309-797)	771	3	314-396 314-449 314-453
5	Mid1 (amino acids 453-797)	938	6	457-529 457-560 457-578 458-560 C467H 458-560 S509G 458-559 488-560
Total	-	27614	17	-

Table S2. Hydrodynamic properties of Mid1p constructs.

Mid1p construct	Concentration (μM)	Sedimentation coefficient (S)	Diffusion coefficient (D) ($10^{-7} \text{ cm}^2 \text{ s}^{-1}$)	Molecular weight (KDa)	Order of oligomerization
M1-12	8.7	2.1	4.75	37.7	1.1
	17.4	2.1	5.34	34.2	1.0
	69.4	2.1	4.97	36.3	1.0
M1-13	4.6	14.6 ± 0.2	3.97 ± 0.89	334.0 ± 69.2	6.5 ± 1.4
	23.0	14.5 ± 0.1	3.48 ± 0.31	367.4 ± 31.8	7.2 ± 0.6
	46.0	14.4 ± 0.1	3.18 ± 0.11	397.6 ± 14.7	7.8 ± 0.3
M1-124	4.2	1.3	1.61	7.4	0.6
		2.0	5.61	31.8	
	21.1	1.6	7.77	18.1	1.0
		2.2	3.72	51.2	
	42.1	1.6	6.86	19.6	0.9
		2.2	4.19	45.6	

Hydrodynamic properties were estimated using SEDFIT analysis of sedimentation profiles of Mid1p constructs in sedimentation velocity (SV) analytical ultracentrifugation experiments. Conditions (unless otherwise stated): 20 °C in buffer containing 20 mM Tris, 150 mM NaCl, 1 mM TCEP pH 8.0. Assumptions: M1-12 and M1-13 sediment as single discrete species, and M1-124 sediments as two discrete non-interacting species. Data on M1-12 and M1-124 represent the average among results from 2 independent experiments at 42,000 rpm. Data on M1-13 represent average \pm SD among results from 5 independent experiments: two at 42,000 rpm (buffer pH 8.0), two at 35,000 rpm (buffer pH 8.0) and one at 28,000 rpm (buffer pH 7.3). Orders of oligomerization (n) were calculated from molecular weights divided by subunit molecular weights calculated from amino acid compositions. Orders of oligomerization were estimated only for the larger of the two M1-124 species at each concentration.

Table S3. Contributions of Mid1p domains to cell cycle time.

Genotype	Number of cell separation events per cell in 5 h	Number of cells scored
Wild type	1.0	42
<i>Δmid1</i>	0.7	51
M1-Δ1	1.0	24
M1-Δ2	0.6	43
M1-Δ3	0.9	31
M1-Δ4	0.5	33
M1-Δ5	1.0	27
M1-Δ6	0.5	48

The number of cell separation events per cell was measured from 5 h of DIC images taken every 2 min, by dividing the number of daughter cell separation events by the number of cells present at the start of image acquisition (the number of cells scored). Genotype of cells used: wild type cells; *Δmid1* cells; *Δmid1* cells expressing constructs from the *mid1* locus: M1-Δ1-2mEGFP, M1-Δ2-2mEGFP or M1-Δ6-2mEGFP; *Δmid1* cells expressing constructs from a cloned *mid1* promoter in the *leu1* locus: M1-Δ3-mEGFP, M1-Δ4-mEGFP or M1-Δ5-mEGFP.